Effect of Microbial Inoculants on the Growth of Silver Oak *(Grevillea robusta)* in Nursery Condition

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***Abstract*—Silver oak belongs to the family Proteaceae, native of Australia but is found abundantly in India. It is used as timber, wind breakers, shade tree, as stake for the climbers and making Cricket bats. Realizing these importance and demand for the production of quality planting stock, a green house experiment was conducted at College of Forestry, Ponnampet, Karnataka, India, to evaluate the performance of microbial inoculants *viz.*, *Trichoderma*, P-solubilizer and N-fixer for growth promoting activity of Silver oak. The experiment was conducted in Completely Randomized Design with ten treatments and three replications. Height, girth and total number of leaves of the plants were recorded at 30, 60, 90 and 120 days after transplanting. The growth of the plants was significantly different between the treatments in all the growth stages for different parameters studied except for number of leaves. The leaves number decreased in later stages *i.e.* 120 days after transplanting. This may be due to the abiotic factors like Temperature, RH *etc.* This study suggests that the potting mixture has to be provided with bioinoculants *viz, Trichoderma,* P-solubilizers and if necessary the Nitrogen fixers based on the nutrient status of the potting mixture and the plant requirement.**

***Index Terms*—P solubilizer, N Fixer, Silver oak, Trichoderma.**

I. Introduction

Industrialization and population explosion in India are the two major drawbacks confined to reduction in agricultural productivity. To increase the agricultural productivity to meet the needs of global population, there should be a change in the current agricultural practices. The high usage of agrochemicals has made soil infertile, accumulation of toxic chemicals in the soil and food products and imbalanced nutrient cycling and ecosystem also occur. In order to maximize the agricultural productivity with minimum soil loss, a cheap, better and safest way is necessary. All these criteria can be achieved through application of microbial bioinoculants. Because, these microorganisms are known to possess vast range of capabilities by producing growth promoting substances, enhancing the plant nutrients, biological N fixation and crop protection against stress and diseased conditions. These microbial inoculants have been shown to cause very real and positive effects when matched correctly to the right plant and the right environmental condition.

Silver oak belongs to the family Proteaceae, the tree is native of east coast of Australia but is found growing in abundance in India. The Silver Oak trees can grow up to 25 to 35 meters tall. The flowers are the most fascinating part of this tree, they are extremely unusual. The flowers are golden orange washing brush like blooms and are toxic. Silver Oak trees is used as a Timber, for making Cricket bats, Used as a wind breakers and it used as a shade tree for plantations and as a stake for the climbers like pepper. Though silver oak is fast growing species, its growth in India is not as fast as in Australia. In spite of application of chemical fertilizers its growth is not as expected. Many different microorganisms used as biofertilizers have both direct and indirect effects in the plant growth promotion, nitrogen fixation, solubilization of phosphate, biocontrol etc. Boosting the nursery growth has an impact on early establishment, higher growth and production of quality planting stock of this commercially important tree species and also reduces the cost of production. Therefore, realizing the importance of this, Bioinoculants study was conducted in order to know its effect on growth of silver oak at nursery level.

II. Materials and Methods

A green house experiment was conducted at College of Forestry, Ponnampet, which is a high rainfall area and a constituent college of University of Agricultural Sciences, Bangalore which is located in the southern parts of Karnataka state, India. To evaluate growth promoting activity of *Trichoderma*, P solubilizer and N fixers singly and in combination on the growth of Silver oak. Bioinoculants such as *Trichoderma, Baccilus coagulans* (P solubilizer) and *Azatobacter* (N Fixer) were obtained from the Dept. of Agricultural Microbiology, UAS, GKVK, Bangalore. One month Silver oak seedlings were procured from a nursery in Chikkamagalore, Karnataka state, India. Potting mixtures were prepared by mixing sand, soil and FYM at 1:1:1 ratio and one kg of the potting mixture was filled in 2000 polythene covers.

One Silver oak plant was planted in each polythene bag.

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Totally one thousand bags were planted and maintained for one month with proper watering and weeding. The experiment was conducted in Completely Randomized Design (CRD) with ten treatments and three replications. Three Hundred seedlings were transplanted at thirty per each treatment having three replications at 10 plants per replication. Mass multiplication of the microbial inoculants was done by using specific growth medium, *viz.,* Potato dextrose medium (PDB) for *Trichoderma* and Nutrient broth for *Bacillus* and *Azatobacter.* Initially the pure culture of *Trichoderma* was inoculated to 100 ml sterilized PDB broth and kept for incubation for six days at 26 ± 2^0C. After the incubation period, the broth culture was inoculated to one liter sterilized PDB and incubated for six days at 26 ± 2^0C with regular shaking of the culture medium. The same procedure was repeated for Bacillus and *Azatobacter* by using Nutrient Broth medium. The mycelial mat of *Trichoderma* was separated by muslin cloth, macerated in sterile water in a warring blender. The *Azatobacter* and *Baccilus coagulans* suspension was prepared by thorough shaking of the broth and the inoculants were poured at 10 ml per plant. Chemical fertilizer like urea, rock phosphate and murate of potash was mixed at 1:1:1 ratio and applied at 5g/ plant. Neem cake was applied at 10 g/ plant. This recommendation of nutrients is commonly practiced in the nursery condition at College of Forestry, Ponnampet.

Plant height of Silver oak was measured from the base to the shoot tip of the seedling at 30, 60, 90 and 120 days after transplanting. Stem girth was measured at the color region of the main shoot using Vernier calipers at 30, 60, 90 and 120 days after transplanting. The girth was calculated using the formula,

Girth = (Main Scale reading + Vernier scale reading) X least count.

Total number of leaves was counted at 30, 60, 90 and 120 days after transplanting.

Population studies of *Trichoderma, Bacillus coagulans* and *Azatobacter* were conducted using serial dilution plate technique method at different intervals [1] Ten grams of rhizospher soil from the polythene covers was taken and it was added to 90 ml water blank to make 1:10 dilution (10^{-1}). 10 ml of the suspension from 10^{-1} was transferred to another flask containing 90 ml water blank to make 1: 100 (10^{-2}) dilution. Further dilutions *viz.*, 10^{-3} and 10^{-4} was prepared by pipetting 10 ml of suspension into additional water blanks as prepared above. One ml aliquots were transferred each from 10^{-3} and 10^{-4} dilutions into three sterile Petri plates aseptically. Approximately 15 ml of the cooled specific culture medium *viz., Trichoderma* specific medium [2], *Bacillus coagulans* specific medium [3] and Waksmann No. 77 medium for *Azatobacter* were poured into the Petr plates and the inoculum were spread by gentle rotation of the petriplates. Upon solidification of the media, the plates were incubated at 26 ± 2^0C for 6 -7 days. The colonies were observed after the incubation. The colonies were counted and population was expressed in cfu X 10^3 /10 g of soil for *Trichoderma* and cfu X 10^4 / 10 g of soil for *Bacillus coagulans* and *Azatobacter.* The data collected in this experimental study were subjected to statistical analysis suitable for completely randomized experiment [4].

III. Results and Discussion

Microbial inoculants have been advocated to provide benefits to growing plants in terms of direct promotion of vegetative growth through atmospheric N fixation, P solubilization and release of growth promoting substances in the rhizosphere which alter root physiology [5]. The present study was aimed to assess the effects of *Bacillus coagulans, Azatobacter* and *Trichoderma* isolates on plant growth of silver oak in the nursery condition. Table I, II and III show the results of height, girth and number of leaves at different growth stages of silver oak respectively.

The data pertaining to the height of silver oak showed an increase at all the stages studied and there was a significant difference between the treatments. Maximum height was observed in *Trichoderma* inoculated plants (17.91 cm) and minimum was in Neem cake inoculated plants (9.60 cm) initially. At 30 DAT the same trend was observed, maximum was in *Trichoderma* inoculated plants (22.42 cm) and minimum was in Neem cake inoculated plants. At 60 DAT there was some difference in the results. Maximum was in *Trichoderma* + *Bacillus coagulans* (30.37 cm) and minimum was in control plants. The same trend was observed at 90 DAT, maximum in *Trichoderma* + *Bacillus coagulans* (32.20 cm) and minimum in control plants (21.01 cm). At 120 DAT maximum and minimum heights were 33.47 cm and 22.03 cm in *Trichoderma* + *Bacillus coagulans* and controlled plants respectively.

TABLE I: Effect of Microbial Inoculants on the Height (Cm) of Silver Oak Seedlings.

Treatments	Height (cm)				
	Initial	30 DAT	60 DAT	90 DAT	120 DAT
T_1 – *Trichoderma*	17.91	22.42	27.80	29.01	29.27
T_2 – *Bacillus coagulans*	14.55	20.89	26.67	26.77	28.53
T_3 – *Azatobacter*	14.23	19.35	25.47	26.08	28.12
T_4 - *Trichoderma* + *Bacillus*	14.90	21.52	30.37	32.20	33.47
T_5 - *Trichoderma* + *Azatobacter*	12.88	19.20	27.27	27.72	29.63
T_6 - *Bacillus* + *Azatobacter*	13.29	18.57	26.33	27.70	28.67
T_7 - *Trichoderma* + *Bacillus* + *Azatobacter*	11.64	18.03	25.12	26.03	27.93
T_8 – Chemical Fertilizer	10.93	15.03	21.35	24.13	24.57
T_9 – Neem Cake	9.60	14.29	20.70	23.08	23.43
T_{10} – Control (Only potting mixture)	10.55	14.47	19.03	21.01	22.03
***F* – Value**	*	*	*	*	*
S.E.	**0.473**	**0.849**	**1.585**	**1.460**	**2.830**

TABLE II: EFFECT OF MICROBIAL INOCULANTS ON THE GIRTH (MM) OF SILVER OAK SEEDLINGS.

Treatments	Girth (mm)				
	Initial	30 DAT	60 DAT	90 DAT	120 DAT
T_1 – *Trichoderma*	2.95	3.05	3.44	3.80	4.38
T_2 – *Bacillus coagulans*	2.73	2.82	2.92	3.38	3.89
T_3 – *Azatobacter*	2.66	2.93	2.97	3.64	3.78
T_4 - *Trichoderma* + *Bacillus*	2.66	2.71	2.76	3.90	4.18
T_5 - *Trichoderma* + *Azatobacter*	2.32	2.40	2.50	3.31	3.93
T_6 - *Bacillus* + *Azatobacter*	2.35	2.51	2.63	3.42	3.67
T_7 - *Trichoderma* + *Bacillus* + *Azatobacter*	2.22	2.36	2.49	3.10	3.78
T_8 – Chemical Fertilizer	2.01	2.21	2.41	3.01	3.77
T_9 – Neem Cake	1.84	2.01	2.22	2.97	3.54
T_{10} – Control (Only potting mixture)	2.27	2.33	2.71	3.37	3.74
***F* – Value**	*	*	*	*	NS
S.E.	**0.098**	**0.114**	**0.108**	**0.108**	**0.420**

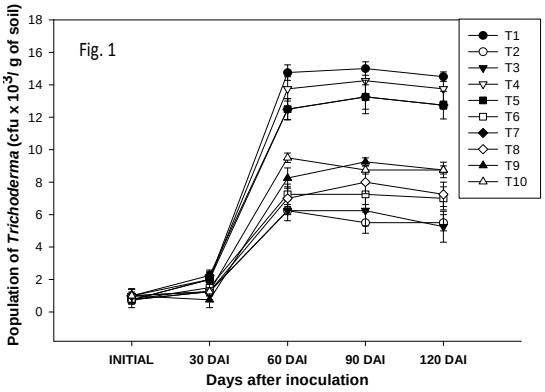
TABLE III: EFFECT OF MICROBIAL INOCULANTS ON THE NUMBER OF LEAVES OF SILVER OAK SEEDLINGS

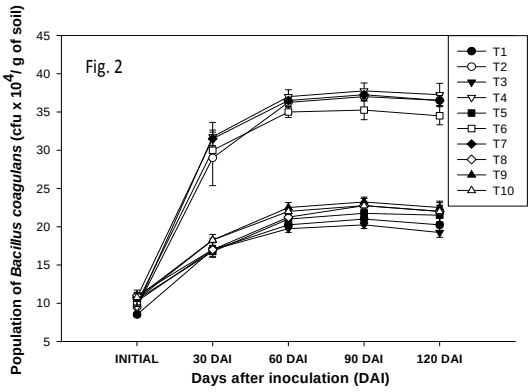
Treatments	Number of leaves				
	Initial	30 DAT	60 DAT	90 DAT	120 DAT
T_1 – *Trichoderma*	2.92	25.25	26.67	20.67	15.38
T_2 – *Bacillus coagulans*	20.17	24.33	27.00	25.58	20.17
T_3 – *Azatobacter*	18.42	22.67	24.00	22.50	17.67
T_4 - *Trichoderma* + *Bacillus*	21.33	25.33	28.08	24.50	21.17
T_5 - *Trichoderma* + *Azatobacter*	19.33	21.92	24.50	25.42	19.58
T_6 - *Bacillus* + *Azatobacter*	18.00	23.33	24.08	24.75	21.17
T_7 - *Trichoderma* + *Bacillus* + *Azatobacter*	18.17	21.75	22.92	22.92	18.75
T_8 – Chemical Fertilizer	18.00	21.08	21.92	23.25	18.25
T_9 – Neem Cake	19.42	24.50	26.25	25.50	21.17
T_{10} – Control (Only potting mixture)	20.17	25.08	25.92	22.33	17.92
***F* - Value**	NS	NS	NS	NS	NS
S.E.	**1.580**	**2.021**	**1.828**	**1.828**	**3.040**

The color diameter was maximum in plants inoculated with *Trichoderma* (2.95 mm) and minimum was in Neem cake inoculated plants (1.84 mm) initially. At 30 DAT and 60 DAT the same trend was observed. At 90 DAT the result was slightly different. The maximum girth was seen in *Bacillus coagulans* treated plants (3.90 mm) and it is on par with *Trichoderma treated* plants (3.44mm), the minimum was in Neem treated plants (2.97 mm). There was no significant difference between the other treatments. The result at 120 DAT was similar to that of 90 DAT, *ie.,* the maximum girth was in *Trichoderma* treated plants (4.38 mm) and it is on par with the plants treated with *Trichoderma Bacillus coagulans* (4.18) and minimum was in Neem treated plants (3.54 mm).The results obtained with respect to the number of leaves in this study showed an interesting pattern. In all the stages studied there was no significant difference between the treatments. The maximum number of leaves was observed in plants which received the treatment of *Trichoderma* and *Bacillus coagulans* at 60 DAT (21.17). Initially it was 21.33 in plants inoculated with *Trichoderma* and *Bacillus coagulans* which is maximum compared to all other treatments and there was no significant difference between the other treatments in the study. At 60 DAT in almost all the treatments there was a decrease in the number of leaves. This may be due to the abiotic factors like temperature, relative humidity *etc.* which may alter the plants' physiology and this has to be studied in future. The overall increase in length and girth of the silver oak could be attributed to the release of growth promoting substances and increased nutrient availability by *Bacillus, Pseudomonas* and *Trichoderma*. The growth promoting substances are known to cause enhanced cell division and root development [6]. The results obtained during the current investigation uphold the results observed by Daiho and Upadhyay [7], Bochow [8], Khan *et al.*[9] and Domenech *et al.*[10] where, in a pot experiment, sand maize culture of *Trichoderma* resulted in an increase in the height and better root development of Soybean plants. Application of N fixer *Aztobacter chroococcum* and P solubilizing bacteria *Bacillus megaterium* increased higher growth parameters compared to Chemical fertilizers in *Mathiola incana*, which is a flower crop [11]. Co–inoculation of Nitrogen fixing organism *Azotobacter* and Phosphate solubilizing microorganisms *Bacillus megaterium* showed a significant increase on the growth of Teak and Indian red wood under nursery condition [12], similar obervation was also obesrved by Umashankar *et al.,* [13] and [14].

The population dynamics for *Trichoderma, Baccilus coagulans* (P solubilizer) and *Azatobacter* (N Fixer) were monitored during the study (Fig. 1, 2 &3). In almost all the treatments initially the population was less and slowly the population increased at 30 DAT and 60 DAT. This could be due to the rhizosphere effect. The plants which were treated with *Trichoderma, Baccilus coagulans* (P solubilizer) and *Azatobacter* (N Fixer) and co- inoculated with combination showed the higher population compared to the control and

un inoculated plants, this suggests that when the *Trichoderma*, isolates introduced alone, multiplied well in the rhizosphere because there will not be any cooperation and the growth will be faster. Similarly high population of *Azatobacter* and *Bacillus* was observed in the treatment that received individual inoculation of *Azatobacter* and *Bacillus*. Treatments where *Trichoderma, Azatobacter* and *Bacillus* inoculated combindly also showed an increase in population this might be due to the enrichment of the soil with the microbial inoculants and could also are due to rhozosphere effect. Papavizas [15] also observed better survival and multiplication of *Trichoderma harzianum* in the rhizosphere, where Pea seeds were treated with bacterial antagonists *Bacillus subtillis*. At 120 DAT the population was decreased, this might be because of the exhaustion of the nutrients from the rhizosphere.





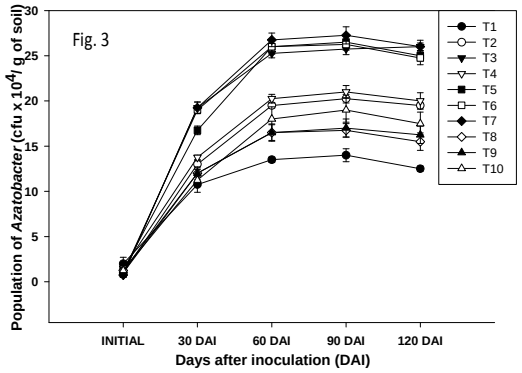


Fig.1, 2 & 3: Populations of *Trichoderma, Bacillus coagulans and Azatobacter* in different growth stages of Silver oak

Many strains of *Bacillus, Pseudomonas* and *Trichoderma* have been implicated in improvement of overall growth of many crop plants [16] - [18]. In the present study, the growth of the plants inoculated with the bioinoculants showed a significant increase over that of the control plants in all the stages studied. Here, the treatment inoculated with *Trichoderma* and *Bacillus coagulans* showed a maximum growth. This indicates that the plant needs additional phosphorous and other plant hormones for its maximum growth which is not provided in sufficient quantity by the potting mixture. The similar study conducted by Sumathi, *et al.* in which the application of *Trichoderma viridae, Bacillus megatherium, Azospirillun lipoferum. Pseudomonas fluorescens* and AM fungi combinedly showed higher growth in Turmeric under tropical nursery condition [19]. In an another study by Pavan on the influence of bioinoculants *viz.,* AM fungi, *Rhizobium, Azatobacter* and phosphate solubilizing bacteria on *Albizia lebbeck*, observed that mixed inoculum performs better than the single inoculum [20].The growth of the plants treated with the chemical fertilizers and Neem cake were almost on par with each other and the performance of the plants treated with the bioinoculants was better than those treated with chemical fertilizers and Neem cake separately.

To conclude, the potting mixture at Forestry college nursery may be provided with biofertilizers *viz, Trichoderma, Bacillus coagulans* and if necessary the *Azatobacter* based on the nutrient status and the plant requirement. The optimum temperature and relative humidity should be standardized and should be maintained in the green house where silver oak plants are grown. This might reduce the dropping of leaves from the plants. Similar experiment can also be conducted outside the green house to find out the reasons for the leaves drop. By the use of bioinoculants we can reduce the cost incurred for growing silver oak in nursery condition.

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